

## AMENDMENTS TO THE SPECIFICATION

**Please replace the fourth paragraph on page 6 of the Specification as filed with the following paragraph:**

Accordingly, another aspect of the present invention contemplates a method for identifying a population of T-cells, said method comprising obtaining a sample comprising CD4<sup>+</sup> T-cells and 'subjecting said CD4<sup>+</sup> T-cells ~~and subjecting said CD4<sup>+</sup> T-cells~~ to surface marker discrimination means on the basis of levels, presence or absence of CMRF-35 epitope and CD45RO marker and optionally CXCR3.

**Please delete the second paragraph on page 8 of the Specification as filed.**

**Please replace the third paragraph on page 6 of the Specification as filed with the following paragraph:**

Figure 23 is a graphical representation showing dot blot analysis. Human peripheral blood T lymphocytes were purified into the CMRF-35<sup>+</sup>CD4<sup>+</sup> and CMRF-35<sup>-</sup>CD4<sup>+</sup> T lymphocyte populations activated with PMA/ionomycin for four hours. GolgiPlug was added for the last 2 hours before cell populations were assayed for intracellular IFN $\gamma$  staining. Results are from one of 3 representative experiments.

**Please delete the fourth paragraph on page 8 of the Specification as filed.**

**Please replace the third paragraph on page 6 of the Specification as filed with the following paragraph:**

Figure 35 is a graphical representation of the intensity of CD95 staining. Human peripheral blood T lymphocytes were purified into the CMRF-35<sup>+</sup>CD4<sup>+</sup> and CMRF-35<sup>-</sup>CD4<sup>+</sup> T lymphocyte populations activated with immobilized CD3/CD28 antibodies overnight. Cell populations were assayed for CD95 staining. Results are from one experiment.

**Please replace the third paragraph on page 6 of the Specification as filed with the following paragraph:**

Figure 46 is a graphical representation of a dot blot analysis. PBMCs from a normal donor and a patient with psoriasis were analyzed using flow cytometry. CD4<sup>+</sup> T cells were stained for the

expression of CXCR3 and CMRF-35. Analysis revealed that patients with psoriasis had significantly reduced levels of CXCR3<sup>+</sup> CMRF35<sup>++</sup> CD4<sup>+</sup> T cells.

**Please replace the third paragraph on page 12 of the Specification as filed with the following paragraph:**

Accordingly, another aspect of the present invention contemplates a method for identifying a population of T-cells, said method comprising obtaining a sample comprising CD4<sup>+</sup> T-cells and subjecting said CD4<sup>+</sup> T-cells to surface marker discrimination means on the basis of levels, presence or absence of CMRF-35 epitope and CD45RO marker and optionally CXCR3<sup>+</sup>CDCR3<sup>+</sup>.

**Please replace the first paragraph on page 13 of the Specification as filed with the following paragraph:**

Accordingly, another aspect of the present invention contemplates a method of identifying a potential or risk of a particular condition being present or developing said method comprising collecting a sample of blood and subjecting the sample to surface marker discrimination means to identify the level, presence or absence of a CD4<sup>+</sup> T-cell population selected from:

CMRF-35<sup>++</sup> CD45RO<sup>+</sup>;  
CMRF-35<sup>+</sup> CD45RO<sup>+</sup>;  
CMRF-35-CD45RO<sup>+</sup> CMRF-35<sup>+</sup> CD45RO<sup>+</sup>;  
and CMRF-35<sup>-</sup>CD45RO<sup>-</sup> T-cells;

wherein an alteration in the levels, presence or absence of one or more of the above T-cell populations is indicative of a disease condition or the propensity for a disease condition to develop. As above, the CMRF-35<sup>++</sup> CD45RO<sup>+</sup> population may also be CXCR3<sup>+</sup>CDCR3<sup>+</sup>.

**Please replace the first paragraph on page 88 of the Specification as filed with the following paragraph:**

The CMRF-35 mAb is described by Daish et al, 1993 Supra. ~~Directly~~-PE, FITC, PerCP or APC conjugated antibodies for CD3, CD19, CD14, CD34, HLA-DR, CD62L, CD49d, CD25, CD4, CD45RO, IgG1 controls were obtained from BD Biosciences (San Jose, CA). CD56-PE, CD28-PE and CD38-PE were obtained from BD Pharmingen. CXCR3-FITC and CCR7-FITC were

obtained from R&D Systems (UK). Sheep anti mouse-PE and FITC conjugates were obtained from Chemicon (Melbourne).

**Please delete the third paragraph, i.e., lines 23-26, on page 88 of the Specification as filed.**

**Please replace the second header and the last paragraph on page 89 of the Specification as filed with the following header and paragraph:**

**Allogeneic Mixed Leucocyte ~~Lueocyte~~ reactions (MLR)**

Lin<sup>-</sup> HLA-DR<sup>+</sup> dendritic cells (DC) were purified from the ER-fraction of the PBMCs using magnetic bead immunodepletion followed by negative selection on the FACS Vantage. ER-cells were labeled with CD14 (CMRF-31), CD19, CD56, CD34. Following washing, the cells were incubated with Biomag goat anti-mouse-immunoglobulin-coated magnetic beads (Polysciences Warrington, PA, USA). Labeled cells were depleted by first pre-clearing with a MCP-1 magnet (Dyna, Oslo, Norway) followed by passing through a MACS CS column on a Variomacs magnet (Miltenyi Biotech, Gladbach, Germany). To obtain highly purified DC, the immunodepleted cells ~~were cells~~ were labeled with CD64-PE, CD20-PE, CD11b-PE and HLA-DR-APC. The cells were sorted by the FACS Vantage and the HLA-DR<sup>+</sup>, Lino population collected.

**Please replace the first paragraph on page 91 of the Specification as filed with the following paragraph:**

Intracellular staining for cytokines was as per the manufacturer's instructions (Fix/Perm Kit, CALTAG LABORATORIES) except for the intracellular labeling incubation step, where samples were incubated at 4°C instead of the recommended room temperature (as per ~~recommendations~~ Pharmingen recommendations for their antibodies). Cells were surface labeled with CD25 using directly conjugated mAb (BD Biosciences). Protein transport was inhibited using Golgi Plug (Trademark; Pharmingen) and stained for intracellular cytokines with IL 10-PE, IL4-PE and IFN-γ-FITC or IFN-γ-PE (Pharmingen).

**Please replace the first paragraph on page 95 of the Specification as filed with the following paragraph:**

A difference in function between the CMRF-35<sup>+</sup> T lymphocyte population and the CMRF-35<sup>-</sup> T lymphocyte population was assessed by the ability of each population to respond to Lin<sup>-</sup>HLA-

DR<sup>+</sup> stimulators in a MLR. The CD3<sup>+</sup>CMRF-35<sup>-</sup> (Figure 2A), the CD4<sup>+</sup>CMRF-35<sup>-</sup> (Figure 2B) or the subpopulations showed a greater proliferative ability than the CD3<sup>+</sup>CMRF-35<sup>+</sup> or CD4<sup>+</sup>CMRF-35<sup>+</sup> populations of T lymphocytes. As the CMRF-35<sup>+</sup> cells were prepared by positive selection, a control was used were mAb labeled cells that had been through the flow cytometer without sorting were compared to the sorted populations and labeled T lymphocytes. No significant difference was seen between the labeled and unlabeled T lymphocytes.

**Please replace the second header and the second paragraph on page 95 of the Specification as filed with the following header and paragraph:**

**EXAMPLE ~~EXAMPLE~~ 13**

**CD3<sup>+</sup> CD4<sup>+</sup> CMRF-35<sup>+</sup> subset is less responsive to in vitro activation signals than the CD3<sup>+</sup> CD4<sup>+</sup> CMRF-35<sup>-</sup> subset**

The stimulator cells in the MLR were Lin-HLA-DR<sup>+</sup> blood DC which are also CMRF-35<sup>+</sup>. In vitro activation of T lymphocytes was used as a single cell system thus removing any influence of CMRF-35 activity on the stimulators. Purified CD4<sup>+</sup>CMRF-35<sup>+</sup> and CD4<sup>+</sup>CMRF-35<sup>-</sup> populations of CD4<sup>+</sup> T lymphocytes were activated in vitro with either PMA/ionomycin or immobilized CD3/CD28 mAb and compared to unfractionated T lymphocytes. The CMRF-35<sup>+</sup> subset incorporated lower levels of [<sup>3</sup>H]-thymidine than the CMRF-35<sup>-</sup> fractions or unseparated fractions (Figure 2B). Activation of the CMRF-35<sup>+</sup> and CMRF-35<sup>-</sup> populations was assessed by upregulation of CD25 and CD69. Whilst all populations appeared to show similar levels of CD69 upregulation after 24 hours (Figure 2C) when treated with PMA/ionomycin or immobilized CD3/CD28 the upregulation of CD25 differed between populations. The CMRF-35<sup>+</sup> population upregulated CD25 to a greater extent than the CMRF-35<sup>-</sup> population following treatment. Activation of the CMRF-35<sup>-</sup> population was similar to activation of the unfractionated T lymphocytes.

**Please replace the first full paragraph on page 97 of the Specification as filed with the following paragraph:**

CMRF-35<sup>+</sup> and CMRF-35-CD4<sup>+</sup>T lymphocytes were activated *in vitro* in the presence of exogenous IL-2. It was noted that As seen in Figure 2, the presence of excess IL-2 did not restore the proliferative capacity of the CMRF-35<sup>+</sup> CD4<sup>+</sup>T lymphocytes to that of the CMRF-35<sup>-</sup> CD4<sup>+</sup> T lymphocytes. Thus the lack of proliferation seen is not due to lymphokine deprivation.

**Please replace the paragraph that spans pages 97-98 of the Specification as filed with the following paragraph:**

*In vitro* activated CMRF-35<sup>+</sup> and CMRF-35<sup>+</sup>CD4<sup>+</sup> T lymphocytes were stained with annexin V-EGFP and propidium iodide and assessed for apoptotic cells. Following *in vitro* activation with either PMA/ionomycin or CD3/CD28, the CMRF-35<sup>+</sup>CD4<sup>+</sup> T lymphocyte population was more susceptible to apoptosis than the CMRF-35<sup>+</sup>CD4<sup>+</sup> T lymphocytes and range between 70-80% of the CMRF-35<sup>+</sup>CD4<sup>+</sup> population compared to 43-63% of the CMRF-35<sup>+</sup>CD4<sup>+</sup> population. The differences in the susceptibility of the CMRF-35<sup>+</sup>CD4<sup>+</sup> T lymphocyte compared to CMRF-35<sup>+</sup>CD4<sup>+</sup> T lymphocyte to undergo apoptosis was apparent by 4 hours when PMA/ionomycin was used to activate the cells (Figure-4). Similarly activation with CD3/CD28 mAb caused an increase in the number of cells undergoing apoptosis in the CMRF-35<sup>+</sup>CD4<sup>+</sup> T lymphocyte population compared to the CMRF-35<sup>+</sup>CD4<sup>+</sup> T lymphocyte population.

**Please replace the second header and the second full paragraph on page 98 of the Specification as filed with the following header and paragraph:**

#### **EXAMPLE 19**

**CMRF-35<sup>+</sup>CD45RO<sup>+</sup>CXCR3<sup>+</sup> T cells are depleted from the peripheral blood of patients with psoriasis**

Using the same method as disclosed in Example 2~~above-identified methods~~, PBMCs were isolated from the peripheral blood of normal donors and patients with psoriasis, and the CD4<sup>+</sup> T cells stained for CMRF-35 and CXCR3. Analysis demonstrated that the CMRF-35<sup>+</sup>/CXCR3 population of cells is significantly reduced in the peripheral blood of patients with psoriasis, compared to normal controls (Figure 6).

**Please replace the first paragraph on page 99 of the Specification as filed with the following paragraph:**

Populations of CMRF-35<sup>Hi</sup>CD4<sup>+</sup>CD45RO<sup>+</sup> peripheral blood cells were ~~were~~ analysed in various disease states. The following diseases were tested: Breast cancer, Multiple myeloma, Non Hodgkin's lymphoma, Rheumatoid arthritis, Thyrotoxicosis, SLE, IgA Nephropathy, Idiopathic Thrombocytopenia ~~Thromboeyopenia~~ Purpura, Hashimoto's thyroiditis ~~throiditis~~, Coeliac

Disease and Graves Graves Disease. There was no similar change in the CD4<sup>+</sup>CD45RO<sup>+</sup>CMRF-35<sup>+</sup> population in any of these ~~diseases~~ diseases as seen for psoriasis.

**Please replace the first paragraph on page 100 of the Specification as filed with the following paragraph:**

The level of binding of the CMRF-35 mAb (MF1) to lymphocytes from psoriasis ~~psoriasis~~ patients shows a great range than those from normal donors (Table 5).